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Histological and ultrastructural characteristics of the thyroid of rats treated with ethanol

M. Bakalska-Necheva, D. Chabane Sari, H. Merzouk**

Institute of Cell Biology and Morphology, Bulgarian Aacademy of Sciences, Sofia
**Institute of Biology, University of Tlemcen, Algeria*

Histological and ultrastructural changes in thyroid of rats after treatment with different doses of ethanol were investigated. The animals were given for 30 days a solution of 10, 20, 30 and 50% of ethyl alchohol instead of drinking water. At the end of the 4th week the body weight of rats treated with 50% ethanol decreased about 30% in comparison with the initial one. The lower doses (10 and 20%) have a stimulating effect on the gland activity. Considerable damages in the thyroid of animals receiving a 50% of ethanol were observed both on histological and electronmicroscopical level. The main ultrastructural changes included reduction of the endoplasmic reticulum, lysis of mitochondria and Golgi apparatus.

Key words: thyrocyte, ethanol, ultrastructural changes.

In the recent two decades there appeared new data about the character and the dynamics of the changes in different endocrine organs under the influence of alcohol. According to contemporary views the hypothalamic-hypophisal axis takes an important place in the mechanism of chronic alcoholism. In this direction a special attention is paid to the investigation on the influence of alcohol on the structure and function of the biomembrane cellular systems resulting in changes of cell metabolism, energetic balance, regulation of cell activity. Still, however, is known about the effect of alcohol on the integrity of the neuroendocrine system. First in this respect is lacking of morphological data. Especially, the investigations on the morphofunctional state of such an important endocrine organ as the thyroid, are poor. There is some information on the basis of clinic biochemical data about the contents of thyroid hormones in blood. According to Israel et al. [10] in cases of alcoholic liver disease the concentration of T_3 and T_4 in blood serum significantly decreases. Lower is the level of TSH too [11]. An expressed hypothyroidism and a decreased autoimmune response is observed in 64% of the patients with chronic alcoholism [5].

The purpose of this study is to analyse the histological and ultrastructural changes in the rat thyroid after treatment with ethanol during 30 days.

Material and methods

The experiments were performed on white 2 months old rats Wistar. The animals were given a solution of ethanol in different concentrations instead of drinking water. They were divided into the following experimental groups:
1st group — rats treated with 10% solution of ethanol;
2nd group — rats treated with 20% solution of ethanol;
3rd group — rats treated with 30% solution of ethanol;
4th group — rats treated with 50% solution of ethanol.
Rats of corresponding age bread in normal conditions were used as controls. The body weight of the animals was measured daily and at the end of each week the average values were calculated. At the 30th day all the animals were killed and pieces of the thyroid were taken for histological and electronmicroscopic studies. The material for histological observation was fixed in Bouin's mixture and included in paraffin. On serial sections of the gland, stained with haematoxilin-eosine, the following morphometric investigations were carried out: in a 100 follicles the height of 10 opposite thyrocytes was measured by an ocular micrometer and the average value was calculated. The percentage ratio of the theree basic structural components of the gland: thyroid epithelium — colloid — connective tissue was measured and the results were statistically processed. For the transmission electron microscopy (TEM) small pieces of the glands were fixed in 2,5% glutaraldehyde, post-fixed in 1% OsO_4 and then processed further by the routine method. The observations were made with EM "Opton-109".

Results and discussion

On Table 1 the average values of the body weight of the animals of the corresponding groups were shown. In rats treated with 10, 20 and 30% ethanol the weight similar to that in control animals increases progressively in the course of the experiment. Differences were found only for the animals treated with 50% ethanol. From the second week a diminution in the weight of these rats began and at the end of 4th week it decreased with 30% in comparison with the initial one.

Histological study. The histological analysis of the thyroid sections of the control animals (Fig. 1 — *A*) showed the domination of follicles of middle size with cubic thyrocytes. The larger follicles were situated peripherally. The average height of the thyroid cells is 4,09 μm. The percentage ratio of thyroid epitelium: colloid: connective tissue is respectively 41,16: 49,83: 8,66 (Table 2). The colloid is of a pale pink colour. All these morphological characteristics suggested a moderate functional activity of the organ. The study of the thyroid from the experimental rats treated with a 10% alcohol revealed the following changes. In most of the follicles the colloid was more clear with the appearance of the zones of re-

Table 1. The average values of the weight of the rats, g

Group	S_0	S_1	S_2	S_3	S_4
1st—10%	77,25± 5,90	90,35±13,93	117,65±20,83	151,25±21,11	178,20±19,64
2nd—20%	83,60± 4,91	88,47± 6,18	102,95± 9,29	125,12± 4,66	151,20±14,85
3rd—30%	96,83±15,57	95,50±16,48	104,46±19,71	121,90±25,83	143,10±34,59
4th—50%	118,16± 8,26	103,93±10,27	92,26±14,68	85,30±15,30	84,30±17,93
Control rats	68,40± 2,27	84,45± 7,57	95,17±12,36	131,82±13,57	155,80±20,49

Note: S_0 — inital weight; S_1—S_4 — body weight at the end of the 1st to 4th week respectively.

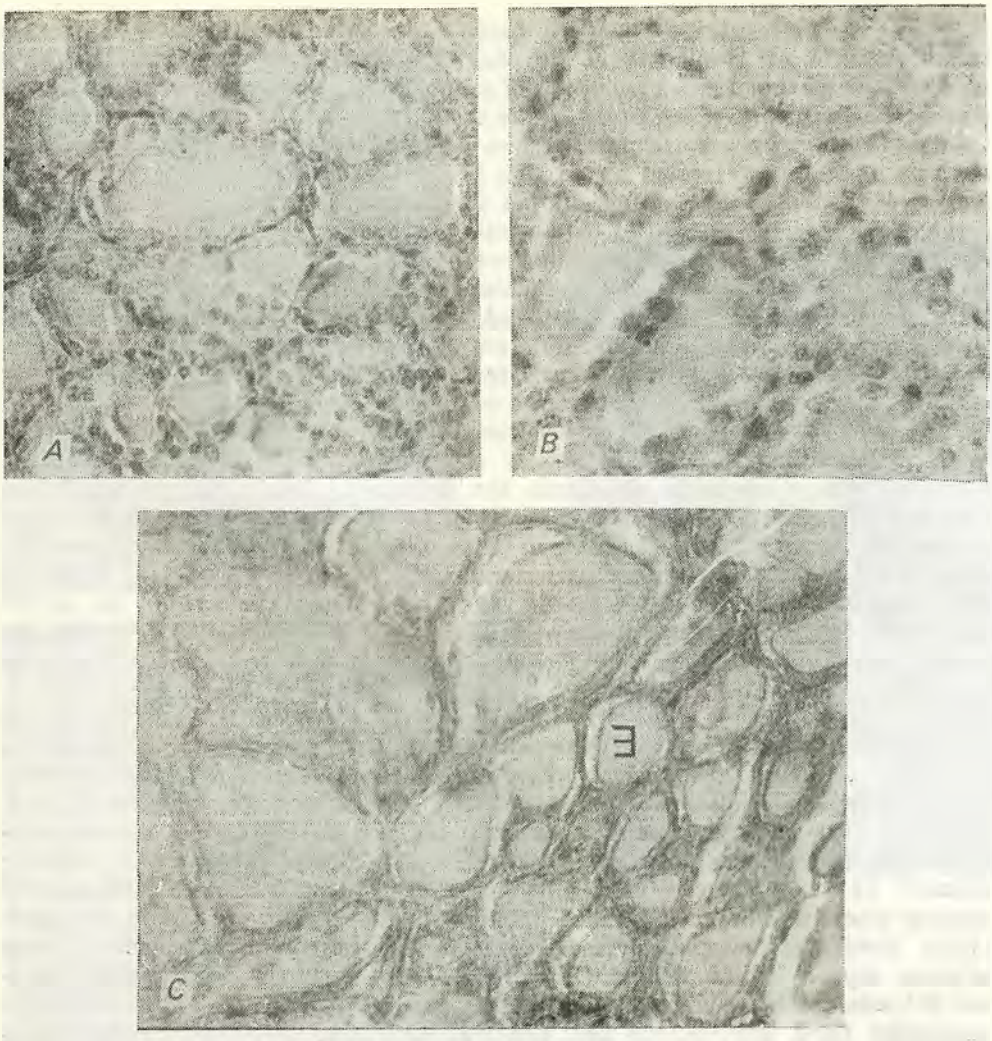

Fig. 1. Histological sections of the rat thyroid. Haematoxilin-eosine staining
A — control rat, × 250; *B* — rat treated with a 30% solution of ethanol, × 300; *C* — rat treated with a 50% solution of ethanol (Э), × 250

Table 2. The percentage ratio of thyroid components

Group	Thyroid epithelium	Colloid	Connective tissue
1st—10%	57,00±7,93	33,33±10,25	10,22±3,48
2nd—20%	57,11±4,09	36,00± 4,32	7,33±1,33
3rd—30%	58,00±3,16	33,00± 2,44	9,00±3,54
4th—50%	33,22±2,18	57,77± 2,65	9,00±2,29
Control rats	41,16±7,38	49,83± 6,84	8,66±6,54

sorption. The thyrocytes, prismatic in form, had a light apical part. Their average height was 5,11 μm. Similar is the morphologic picture of the thyroid of rats treated with 20% ethanol. Follicles in a state of an increased functional activity were found even in the periphery of the organ where resting follicles are usually situated. The values of the morphometric measurements of the three components were near to those of the group treated with a 10% (Table 2), they are another proof on the active resorption of the stored thyroid hormones. The alterations began more demonstrative in the thyroid of the animals treated with 30% ethanol (Fig. 1—*B*). The percentage of the thyroid epithelium reached its maximim — 58% and the colloid was 33%. In some follicles an invagination of the thyrocytes and a modification of the follicle wall were found. The histological picture in the thyroid of the rats treated with 50% ethanol was very different that of the other experimental animals. Most of the follicles were in an inactivated functional state. The thyrocytes were low to flat (Fig. 1—*C*). Their height was significantly decreased — 3,21 μm; the thyroid epitelium — 33,22%. Follicles with dense stored colloid were dominant. In some cases a desquamation is observed — e. g. a penetration of fragments or whole epithelial cells into the lumen. A destruction of the follicle wall and of entire cytoarchitectonic of the gland was preset. The comparison of the morphometric parameters (Table 2) showed essential changes in the percentage ratio of the thyroid epithelium. It is significantly higher in the group, receiving 10%, than in the control group. These values were almost the same in the second and in the third experimental group — 57% and 58% respectively. For the animals, treated with 50% they were lower — 33,33%. The parameters of the colloid were reciproc.

Electronmicroscopic study. The thyrocytes of the control rats (Fig. 2—*A*) were characterised by an well-expressed polarity, a great number of microvilli of the apical surface and an ultrastructure, similar to that of the most secreting cells (developed granular endoplasmic reticulum, presence of free ribosomes, Golgi apparatus, secretory granules of different size and density and numerous mitochondria). The nucleus, spherical in shape, with 1-2 small nucleoli was centrally situated. The ultrastructure of the thyrocytes of rats treated with 30% ethanol showed essential differences related to the plasmolem, as well as to the cytoplasm itself. On the apical surface the microvilli were short, fragmented, most of them dipped in the colloid (Fig. 2—*B*). Thyrocytes in the animals received 50% ethanol showed more expressed disturbances (Fig. 2—*C*). The cytoplasm is vacuolized, filled with electron light granular substance. The elements of the granular endoplasmic reticulum and Golgi apparatus, as well as the mitochondria were damaged. The nucleus is also deformed. The chromatine is more condensed, the nuclear membrane — disrupted at same places. In the animals treated with a 30% solution of ethanol single cells were damaged, whereas in case of 50% they were more numerous.

The ethyl alcohol has many-sided effect on the organism, especially on the endocrine organs [2, 7, 9]. The degree of the caused damages are dose-dependent. On the other hand, the duration of the treatment is also an important factor. The alterations are based on two phenomena: the direct influence on the cellular biomembranes and the action of neurotransmittors such as acetylcholin and biogenic amines (catecholamines, serotonin, etc.) [1, 3, 4]. Ethanol is able to modify the chemical content, as well as the physical state of the biomembranes [6, 8].Being a liposolvent, its molecules are included in the phospholipid layers of the membranes thus desorganising and modifying their viscosity and permeability. Besides it has a direct influence on the membrane proteins, especially the membrane bound enzymes [8]. For example the K^+ — Na^+ dependent ATF-ase is activated by small doses ethanol, but it is inhibited by the bigger ones [1].

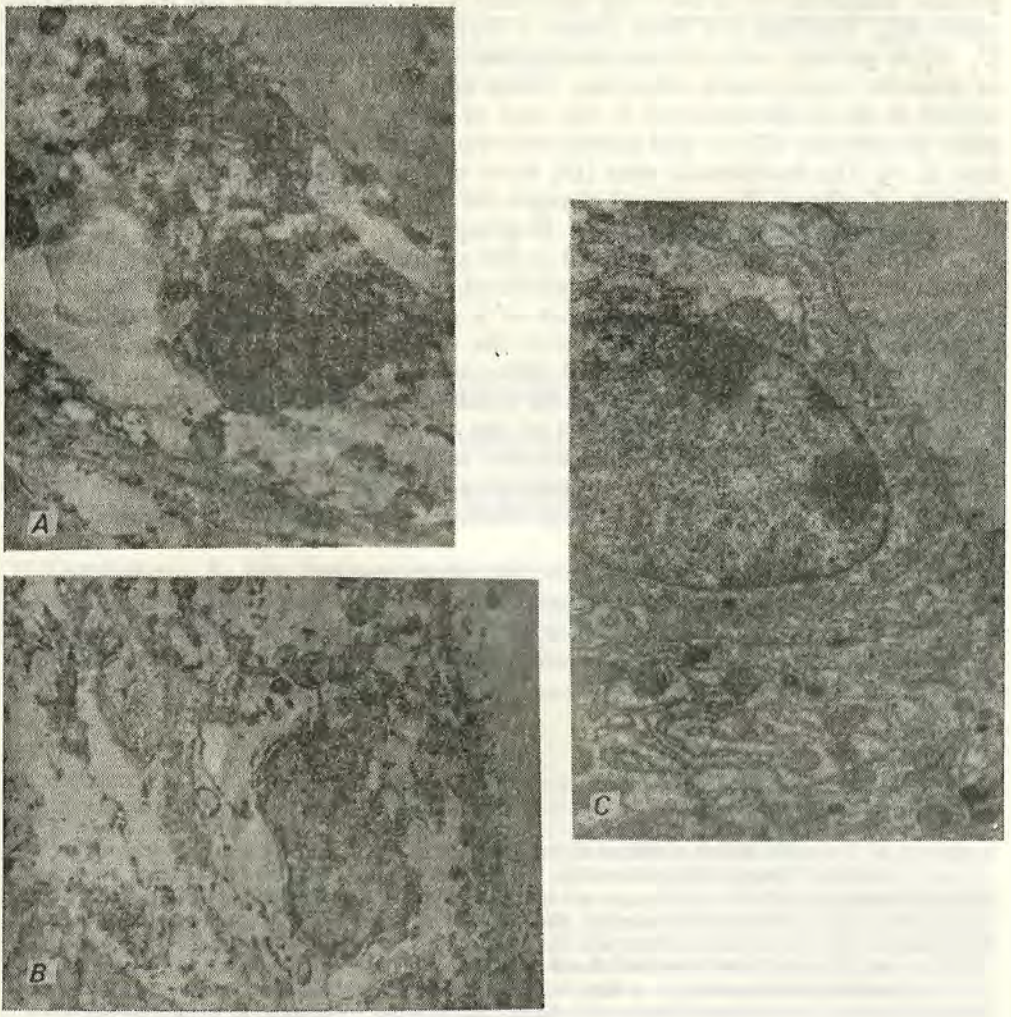

Fig. 2. Electronograms from rat thyrocytes
A — control rat, × 10 000; *B* — rat treated with a 30% solution of ethanol, × 7000; *C* — rat treated with a 50% solution of ethanol, × 7000

The pathogenesis of the alcohol damage of the thyroid is a multifactor process, which includes the direct or indirect participation of the other endocrin organs and regulating mechanisms [11, 16]. The comparison of the histological picture with the morphometric parameters, obtained in the present study shows that the lower doses (10, 20%) administrated in our experiment, have a stimulating effect to a certain degree, on the functional activity of the gland (an increase in the height of the thyrocytes, an activation of the colloid resorption). However, according to results concerning the prolonged application of a diet of 20% ethanol during a 5 months period (from the 10th day on after birth) leads to a functional exhaustion of the rat thyroid, as well as to an essential destruction of almost all cellular organoids. The ultrastructural picture is the same as that observed in the animals receiving a 50% ethanol. Our findings (damage of the granular endo-

plasmic reticulum and of the Golgi apparatus which participate in the thyreoglobulin synthesis), confirm the data about a thyroid hormones defficiency in the blood [5, 10] in patients with chronic alcoholism. Considerable ultrastructural damages of granular endoplasmic reticulum, Golgi apparatus and mitochondria were observed in the cardiomyocytes in the case of alcoholic cardiomiopatia [13, 15]. Similar destruction of liver and kidney cells was reported at acute alcoholic intoxication [2, 9]. The biochemical data [14] show that the acetaldehyde (an intermediate product of ethanol dissociation) changes the protein synthesis by blocking the cellular enzymes interacting with the H-groups.

The colloid resorption, observed in this study, is activated during the treatment with 10 and 20% ethanol, but an inhibition of the process appeared in animals receiving 50% solution. The existence of a colloid-like substance in most of the thyrocytes is probably connected with the alteration of the permeability of the apical plasmolem and with a colloid diffusion into the thyrocytes. From the other side the structural degeneration of the mitochondria decreases the cells energetic potential and disturbs the transport of the colloid which remains stocked in the cytoplasm. Besides, the high concentration of alcohol in blood has a toxic effect on the big blood vessels of the thyroid thus breaking the follicles [5]. The desquamation of thyrocytes and the desorganisation of the follicle wall observed by us supported this data.

On the base of the investigations previously reported and of our results [12], the conclusion that the ethanol causes important structural and metabolic changes in thyroid, can be made. Both similar type of studies and biochemical analysis can help to elucidate the molecular and cellular mechanisms of the alcoholism and its eventual treatment.

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